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Variable Tolerance of the Developing Follicle and Corpus Luteum to Gonadotropin-Releasing Hormone Antagonist-Induced Gonadotropin Withdrawal in the Human*

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ABSTRACT. To examine the differential sensitivity of the ovary to temporary withdrawal of gonadotropin support at different stages of folliculogenesis and corpus luteum function, GnRH antagonist blockade of gonadotropin secretion was examined in 17 studies using the Nal-Glu GnRH antagonist. A vehicle control, antagonist treatment, and follow-up cycle format was used in each study. A previously determined ED_{100} dose of the Nal-Glu GnRH antagonist (150 μg/kg) or vehicle was administered sc every 24 h for 3 consecutive days in the midfollicular phase (MFP), late follicular phase (LFP), and midluteal phase (MLP). In studies in the MFP (n = 7), the largest follicle was 11 ± 2 mm (mean ± SEM), and the mean estradiol (E_2) level was 220 ± 44 pmol/L on the first day of antagonist administration. Administration of the antagonist resulted in a 75 ± 6% suppression of LH ($P < 0.005$), no significant change in FSH, and suppression of E_2 to the assay detection limit ($P < 0.05$). Total cycle length was increased compared to that of the vehicle control cycle (37.3 ± 1.3 *vs.* 26.3 ± 1.1 days; $P < 0.005$) due to prolongation of follicular phase length ($P < 0.005$) and reinitiation of folliculogenesis. In the LFP (n = 5), the largest follicle was 16 ± 1 mm ($P < 0.05$ *vs.* MFP), and the E_2 level was 394 ± 95 pmol/L ($P < 0.05$ *vs.* MFP) on the first day of antagonist administration. Antagonist administration resulted in a 65 ± 6% suppression of LH ($P < 0.05$), a 47 ± 11% decrease in FSH ($P < 0.05$), and no significant change in E_2. Total cycle length was prolonged (32.4 ± 2.2 *vs.* 25.6 ± 0.4 days; $P < 0.05$) due to an increase in follicular phase length ($P < 0.02$); however, the prolongation of the follicular phase was significantly less than that of the MFP (8.0 ± 1.5 *vs.* 15.1 ± 0.1 days; $P < 0.001$), suggesting ovulation from the initial dominant follicle. In studies in the MLP (n = 5), LH, E_2, and progesterone decreased to the assay detection limit after antagonist administration, while FSH decreased by 36 ± 4% ($P < 0.05$). Menstrual bleeding occurred within 24–48 h of the final Nal-Glu antagonist injection. The total cycle length was decreased after antagonist administration (21.8 ± 1 *vs.* 27.8 ± 1.1 days; $P < 0.001$), due entirely to luteal phase shortening (7.2 ± 0.2 *vs.* 14.0 ± 0.7 days; $P < 0.001$) with demise of the corpus luteum.

We conclude that 1) the use of an ED_{100} dose of the Nal-Glu GnRH antagonist for 3 days in normal women results in persistence of the relatively greater suppression of LH compared to FSH previously demonstrated in single dose studies; 2) this degree of gonadotropin deprivation produces different results depending upon the underlying maturation of the dominant follicle or corpus luteum; 3) in the follicular phase, the developing follicle becomes more tolerant to gonadotropin withdrawal as it becomes functionally more mature from the MFP to the LFP; and 4) in the MLP, this degree of gonadotropin withdrawal is not tolerated, and luteolysis occurs. (*J Clin Endocrinol Metab* **72:** 993–1000, 1991)

THE CRITICAL role of GnRH-induced gonadotropin secretion in follicular development and corpus luteum function in the human is undisputed as evidenced by the absence of folliculogenesis in GnRH-deficient women and the creation of normal ovulatory cycles when a physiological regimen of exogenous GnRH is administered to these patients (1). However, evidence suggests that the follicle and corpus luteum vary in their tolerance to gonadotropin withdrawal during their development. While the necessity for gonadotropins in follicular recruitment in the early follicular phase is well established (2), their importance is less clear in the midfollicular phase (MFP). At this time point, the dominant follicle has been selected (2), FSH levels are gradually decreasing in normal women (3), and LH pulse frequency is increasing, but pulse amplitude is significantly blunted (4). By the late follicular phase (LFP), evidence to date indicates that the dominant follicle is controlled to a large extent by local factors which amplify responses to pituitary gonadotropins (5, 6), and few studies have investigated the role of gonadotropins in this stage of the human

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menstrual cycle.

Studies in the primate have suggested that the corpus luteum exhibits a similar variability of gonadotropin dependence, since gonadotropin withdrawal for 24–72 h in the early luteal phase produces only transient decreases in progesterone levels (7, 8). However, by the midluteal phase (MLP), withdrawal of GnRH-induced gonadotropin support in both subhuman primates and women has yielded varying results, with complete luteolysis in some studies but not others (7–10).

To further address the importance of GnRH-induced gonadotropin secretion during different stages of ovarian function, we have blocked GnRH-induced gonadotropin support of the ovary for 72 h with a potent GnRH antagonist at a dose that we have previously shown to suppress LH to greater than 80% and FSH to 40% for 24 h in single injection studies (11). Studies have been performed in the mid- and late follicular phases and the MLP, as these are the cycle stages in which the importance of gonadotropin input to the follicle and corpus luteum remain most controversial. Knowledge of the differential gonadotropin suppression and the degree and time course of ovarian steroid suppression in response to GnRH receptor blockade in different phases of the cycle will have important implications for subsequent clinical applications.

Materials and Methods

Experimental subjects

The study population consisted of 15 euthyroid and normoprolactinemic women, aged 24–42 years (mean ± SEM, 31.4 ± 1.8). Two of the subjects were involved in repeat studies for a total of 17 studies. All subjects had a history of regular menstrual cycles of 25–35 days in length, and ovulation in the cycle before the beginning of the study was confirmed by a MLP plasma progesterone level greater than 11.1 nmol/L. None of the subjects had a prior history of allergy to drugs, and none had used hormonal medications for a minimum of 3 months before the study. A negative intradermal test with the Nal-Glu GnRH antagonist (11) was required before inclusion in the study, and all women agreed to the careful use of barrier contraception during the entire three-cycle study. Ferrous gluconate was administered twice daily for the duration of the study. The study was approved by the Subcommittee on Human Studies of the Massachusetts General Hospital, and informed consent was obtained from each subject before entry.

Protocol

Subjects were studied over three cycles, consisting of a vehicle control cycle, an antagonist treatment cycle, and a follow-up cycle. Blood samples were drawn daily at the same time of day for the first two cycles and weekly for the follow-up cycle for measurement of gonadotropins, estradiol (E_2), and progesterone (P_4). In addition, cycles were monitored by basal body temperature, and the occurrence of vaginal bleeding was recorded. In the two subjects who were studied twice, the initial cycle served as the vehicle control cycle for both studies. In both women, three apparently normal cycles intervened between the follow-up cycle for the first study and the antagonist treatment cycle of the second study, and a MLP progesterone measurement confirmed ovulation in the cycle before the second antagonist treatment cycle. For assessment of toxicity, additional blood samples were drawn for measurement of complete blood count, platelets, liver function, and renal function tests at the beginning of the first and third months of the study.

In the antagonist treatment cycles, a daily dose of 150 μg/kg of the Nal-Glu GnRH antagonist ([Ac-D2Nal[1],D4ClPhe[2],D3Pal[3],Arg[5],DGlu(AA)[6],DAla[10]]GnRH) was administered. The antagonist was formulated in sterile water with 5% glucose (D5W) at a concentration of 10 mg/mL, as previously described (11). This dose has been determined to be an ED_{100} dose, which results in greater than 80% suppression of plasma LH for a duration of at least 24 h in women and a maximal 40% suppression of FSH in single dose studies (11). The antagonist or vehicle (D5W) was administered sc at the same time of day on 3 consecutive days in 1) the MFP, beginning on days 5–9 from menses (*i.e.* days −9 to −5 from expected ovulation) in seven studies; 2) the LFP, beginning on days −5 to −3 from anticipated ovulation, as predicted by serial ultrasound criteria (12), in five studies; and 3) the MLP, beginning 5 days after the urinary LH peak in 6 studies. The subjects were blinded to the order of vehicle control or antagonist cycles; however, the vehicle control cycle always preceded the antagonist cycle, as it was not known whether there would be any carry-over effect from the treatment cycle to the subsequent cycle.

Blood samples were assayed for LH, FSH, E_2, and P_4 by RIA, as previously described (13, 14).

Ovarian ultrasound

Pelvic ultrasounds were performed at frequent intervals throughout the vehicle control and antagonist treatment cycles to assess ovarian size, follicular development, and endometrial thickness. The initial baseline ultrasound was performed transabdominally, so that the entire pelvis could be surveyed; thereafter, all scans were performed transvaginally. The number and sizes of all follicles were recorded from both ovaries as well as the presence of irregular follicular margins or internal echoes.

Data analysis

The day of ovulation was assessed retrospectively from hormonal criteria (4). The follicular phase was counted from the first day of menses to and including the day of ovulation, while the luteal phase was counted from the first day after ovulation until the day before subsequent menses. Total cycle lengths as well as follicular and luteal phase lengths were compared for vehicle control, antagonist, and follow-up cycles using paired *t* tests or Mann-Whitney U tests, as appropriate. In addition, in the two follicular phase groups the length of time from the final antagonist injection to the next ovulation was compared to the follicular phase length in the vehicle control cycle.

To assess the effects of antagonist administration, analysis of the hormonal values was centered to the first day of antagonist injection, and the subsequent changes were expressed in absolute terms and as a percentage of the baseline value immediately before antagonist administration. Percent inhibition from baseline is not reported if the nadir reached was at the limit of detection of the assay. The peak preovulatory E_2, peak P_4, and luteal phase lengths were compared between the control and antagonist treatment cycles for each group using paired statistics. For all studies in the follicular phase (MFP and LFP), the length of time from the final antagonist injection to the next ovulation was correlated with the cycle day, follicle size, and E_2 level on the first day of antagonist administration and with the integrated E_2 up to the time of injection.

The data are presented as the mean ± SEM unless otherwise noted, and $P < 0.05$ was construed as significant.

Results

Vehicle control cycles

In the vehicle control cycles, total cycle length was 27.2 ± 0.9 days; follicular phase length was 14.0 ± 0.8 days; and luteal phase length was 13.6 ± 0.3 days, with no differences among the three study groups. Daily levels of E_2 and P_4 were within the mean ± 1 SD of the range established in our laboratory in 81 normal cycles (1, 4). Consequently, all individual data in the figures use the normal data as a reference for the experimental cycles.

MFP studies

In the MFP studies, the three daily antagonist injections began on days 5–9 after menses (Fig. 1). The follicular size measured within 24 h of the first antagonist injection was 11 ± 2 mm (mean ± SEM) in maximum diameter, and the mean E_2 level was 220 ± 44 pmol/L on the first day of antagonist administration. Antagonist administration was associated with a 75 ± 6% decrease in LH to a nadir at 2 days ($P < 0.005$; Fig. 2A). The decrease in FSH, which was maximum 1 day after antagonist administration (21 ± 10%), was not statistically significant (Fig. 2A). E_2 decreased significantly ($P < 0.05$), with the nadir occurring 2 days after the first day of antagonist administration (Fig. 2A). In all but one subject, E_2 suppressed to the limit of detection of the assay. Although LH and FSH returned to pretreatment levels by 3 days after the final day of antagonist administration, E_2 did not return to pretreatment values until 6 days after the final injection.

The antagonist treatment cycle was significantly longer in total length than the vehicle control cycle (37.3 ± 1.3 *vs.* 26.3 ± 1.1 days; $P < 0.005$; Fig. 3), due entirely to a prolongation of follicular phase length from 13.6 ± 1.2 to 23.9 ± 1 days ($P < 0.005$), with no change in luteal phase length. The number of days from the final antagonist injection until the subsequent ovulation was not different from the length of the vehicle control follicular phase (15.1 ± 0.9 *vs.* 13.6 ± 1.2 days), nor was there a

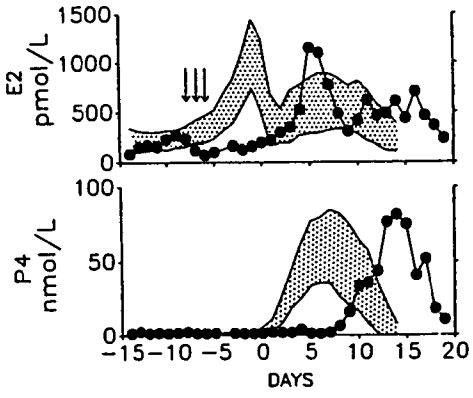

FIG. 1. E_2 and P_4 determinations in a subject who received 150 μg/kg of the Nal-Glu GnRH antagonist daily for 3 consecutive days in the MFP, as indicated by the *arrows*, graphed in relation to the range (±1 SD) determined from 81 normal cycles (*shaded area*), with results centered to the day of ovulation. The subject's results are centered to the day of ovulation in her vehicle control cycle. The follicular phase was prolonged by 7 days compared to her vehicle control cycle.

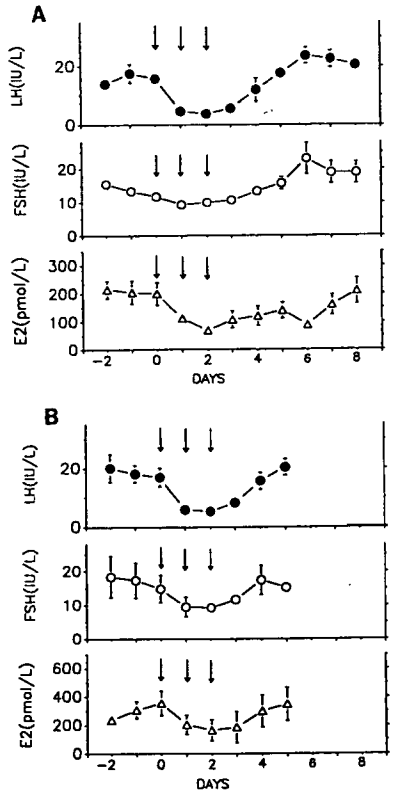

FIG. 2. Mean ± SEM LH, FSH, and E_2 levels from subjects studied in the MFP (A) and the LFP (B). Results are centered on the first day of antagonist administration, as indicated by the *arrows*, and LFP results include all five subjects in this group. See text for discussion of significance. Where not obvious the SEM bars are contained within the symbol.

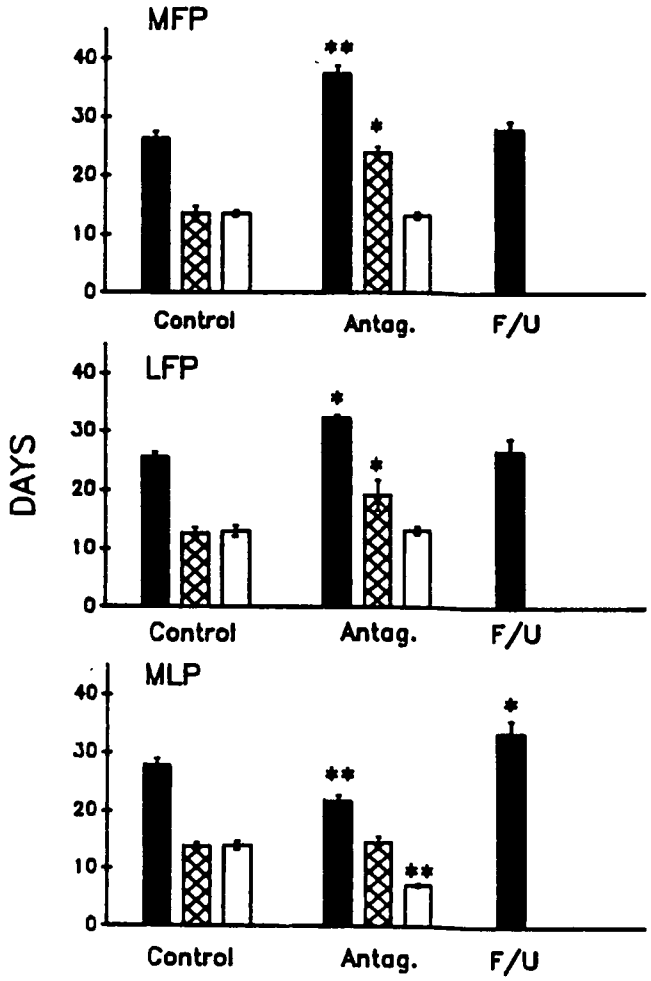

FIG. 3. Total cycle (■), follicular phase (▩), and luteal phase (▭) lengths in the vehicle control, antagonist treatment (Antag.), and follow-up (F/U) cycles, as indicated in the MFP, LFP, and MLP study groups. *, $P < 0.05$; **, $P < 0.005$ (in comparison with the vehicle control data).

difference in the peak preovulatory E_2 or peak luteal phase P_4 in this cycle compared to those in the control cycle. The total length of the follow-up cycle (27.9 ± 1.4 days) was not different from the length of the vehicle control cycle, and all follow-up cycles were ovulatory, as assessed by biphasic basal body temperature (BBT) charts and a plasma progesterone level of 17.8 nmol/L or more.

These changes in hormone concentrations were accompanied by an initial arrest and subsequent decrease in the size of the largest follicle by ultrasound examination. A new dominant follicle appeared in the contralateral ovary in four of the subjects, bilateral dominant follicles subsequently appeared in another two subjects, while one woman was not available for serial follow-up scans. After antagonist administration, there were 2–3 days of vaginal bleeding, beginning 0–3 days from the final injection, in four of the subjects.

LFP studies

In the LFP studies, the first antagonist injection was on days −5 to −3 from anticipated ovulation, as determined by ultrasound criteria (12) (Fig. 4). The mean diameter of the largest follicle on the first day of GnRH antagonist administration was 16 ± 1 mm ($P < 0.05$ *vs.* MFP), and the mean E_2 level was 394 ± 95 pmol/L ($P < 0.05$ *vs.* MFP). LH decreased significantly, with the nadir occurring 2 days after initiation of antagonist treatment ($P < 0.05$; Fig. 2B). This represented a decrease from baseline of 65 ± 6%, not statistically different from the decrease in LH after antagonist administration in the MFP. As opposed to the MFP studies, the decrease in FSH (47 ± 11%) was statistically significant ($P < 0.05$; Fig. 2B), with the nadir occurring at 2 days. The nadir for E_2 levels also occurred at 2 days, but the decrease from baseline was not significant for the group as a whole (Fig. 2B). However, in one of the five subjects, E_2 levels plateaued, rather than decreased, after antagonist administration despite a decrease in LH and FSH that was indistinguishable from the that in the remainder of the group. This subject was distinguished by having two follicles of similar size on ultrasound examination immediately before antagonist administration. When her results were removed from the group, the decrease in E_2 was significant at 2 days ($P < 0.05$, by one-tailed testing). In the LFP studies, LH and FSH had returned to pretreatment levels within 48 h of the final antagonist injection, while E_2 returned to pretherapy levels by 3 days for the group as a whole (Fig. 2B).

In the LFP group, total cycle length was significantly longer in the antagonist cycle than in the vehicle control cycle (32.4 ± 2.2 *vs.* 25.6 ± 0.4 days; $P < 0.05$; Fig. 3),

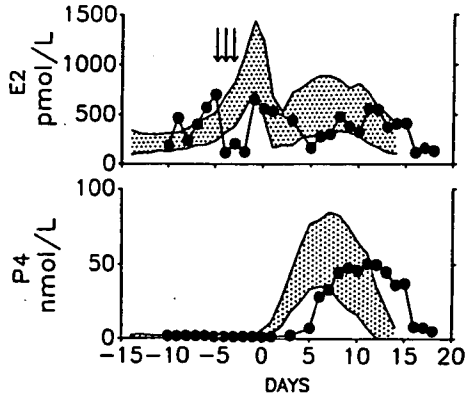

FIG. 4. E_2 and P_4 results in a subject who received the GnRH antagonist (indicated by the *arrows*) beginning 4 days before anticipated ovulation (LFP), graphed in relation to the normal range (*shaded area*). The subject's results are centered to the day of ovulation in her control cycle; the follicular phase was prolonged by 3 days relative to her vehicle control cycle. Two dominant follicles were seen on ultrasound, presumably accounting for E_2 levels that were slightly higher than normal in this subject.

due to an increase in follicular phase length from 12.6 ± 1 to 19.2 ± 2.5 days ($P < 0.02$). However, the length of time to subsequent ovulation after antagonist administration (8.0 ± 1.5 days) was significantly less than the control follicular phase length ($P < 0.001$) and was also significantly less than the time to subsequent ovulation in the subjects who received the antagonist in the MFP ($P < 0.001$). The subsequent preovulatory peak E_2 level was not different from that in the vehicle control cycle, nor was there a difference in peak luteal phase P_4 or luteal phase length. The length of the follow-up cycle was also not different from the vehicle control cycle length, and ovulation in the follow-up cycle was documented by a biphasic BBT and a P_4 of 15.9 nmol/L or more in all subjects.

In this group, serial ultrasound examinations identified slowed progress of follicular growth, but eventual ovulation from the same ovary in one subject and bilateral follicular development and subsequent apparent corpus luteum formation in two subjects. The subject whose E_2 levels plateaued, but did not decrease, had bilateral follicles of similar diameter, one of which subsequently formed a corpus luteum on ultrasound, while the other remained clear and regular, suggesting that it did not ovulate.

Follicular phase correlations

When the 12 studies from the follicular phase were combined, the time to subsequent ovulation from the final day of antagonist treatment was negatively correlated with the E_2 level on the first day of antagonist administration ($r = 0.57$; $P < 0.05$), but not with the cycle day, the follicle size on the first day, or the integrated E_2 levels up to the first day of antagonist administration. These results suggest that a functionally more mature follicle is more resistant to the effects of short term gonadotropin deprivation.

MLP studies

In the MLP subjects, the three daily antagonist injections began on day 4 or 5 after ovulation, as determined by retrospective hormonal analysis (Fig. 5), and an apparent corpus luteum was present on ultrasound before antagonist administration. From the first day of antagonist administration, LH decreased to a nadir at 1 and 2 days (72 ± 7% decrease from baseline; $P < 0.05$; Fig. 6), and FSH decreased 36 ± 4% from baseline at 1 day ($P < 0.05$; Fig. 6). E_2 and P_4 levels decreased dramatically after antagonist administration ($P < 0.01$ and $P < 0.001$, respectively; Fig. 6). Menstrual bleeding occurred within 24–48 h of the final day of antagonist administration in all subjects.

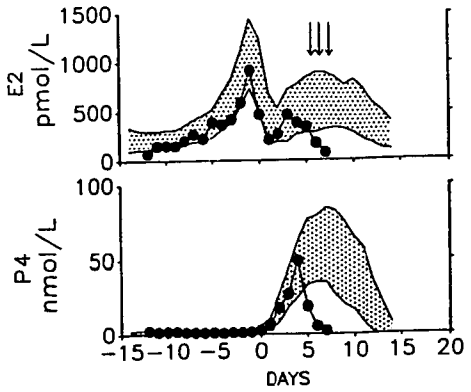

FIG. 5. E_2 and P_4 results in a subject who received the Nal-Glu GnRH antagonist in the MLP on the days indicated by the *arrows* in relation the normal range (*shaded area*). Her results have been centered to the day of ovulation in the antagonist cycle. In this individual, the luteal phase was 5 days shorter than in her vehicle control cycle.

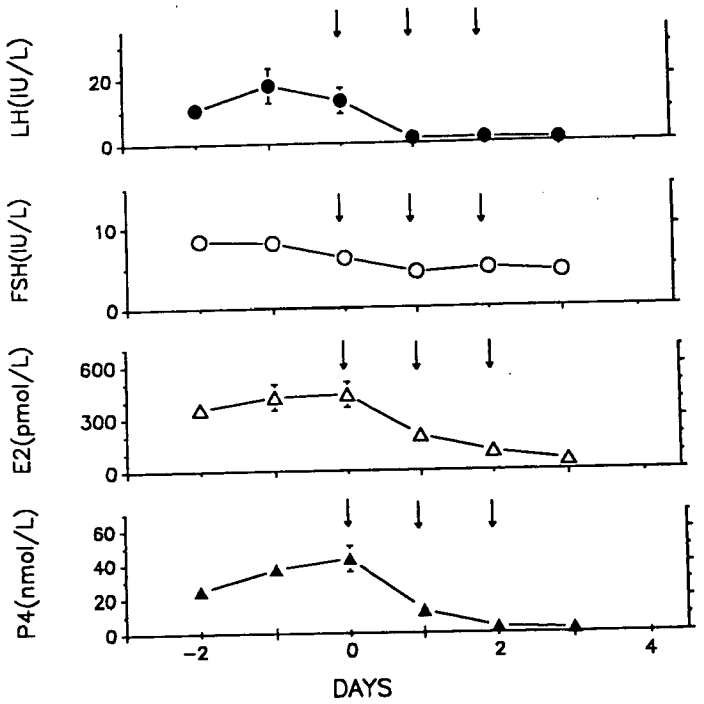

FIG. 6. Mean ± SEM LH, FSH, E_2, and P_4 values of the subjects who received the Nal-Glu GnRH antagonist in the MLP. Results are centered to the first day of antagonist administration, as indicated by the *arrows*. Where not obvious, the SEM bars are contained within the symbol.

The antagonist treatment cycle was significantly shorter in total length than the vehicle control cycle (21.8 ± 1.0 *vs.* 27.8 ± 1.1 days; $P < 0.001$; Fig. 3) due to a decrease in luteal phase length in the antagonist cycle compared to the control cycle (7.2 ± 0.2 *vs.* 14.0 ± 0.7 days; $P < 0.001$). The peak P_4 level was decreased in the antagonist cycle (15.1 ± 1.5 *vs.* 22.4 ± 2.6 ng/mL; $P < 0.04$), but the P_4 increase up to the time of antagonist administration was not different from that in the vehicle

control cycle. Although the follicular phase length was not different in the antagonist compared to the control cycle, the peak preovulatory E_2 levels were higher ($P < 0.01$). The total length of the follow-up cycle (33.6 ± 2.1 days) was somewhat longer than that of the vehicle control cycle ($P < 0.04$), but hormonal values were not available to determine whether this difference could be attributed to an increase in follicular or luteal phase lengths. BBT charts were biphasic in all follow-up cycles, and hormonal levels from weekly blood samples were indicative of ovulation in four subjects and showed a LH peak in the fifth.

Toxicity

There was no significant change in hemoglobin, white blood cell count, platelet count, or any indices of renal or hepatic function after daily administration of the Nal-Glu GnRH antagonist for 3 consecutive days. Subcutaneous injection of this antagonist resulted in local erythema, induration, and mild to moderate discomfort at the injection site in all cases at the dose used in these studies. The erythema was transient and had usually resolved within 30 min. The induration had generally resolved by the time the subject was examined 24 h later, but in two women a small nodule (5 × 5 mm) was observed to last 3–4 weeks. There were no systemic side-effects observed in these studies. One subject became pregnant in the cycle after the follow-up cycle and has since delivered a healthy infant.

Discussion

These studies indicate that the extent to which 72 h of gonadotropin deprivation induced by GnRH receptor blockade influences ovarian function depends on the time of the cycle in which it occurs. Several lines of evidence indicate that in subjects who received the GnRH antagonist in the MFP, the initial dominant follicle regressed, and folliculogenesis was reinitiated. In four of the seven subjects in this group and four of the five in which an accurate assessment could be made, there was clear evidence of a new dominant follicle on the side opposite that seen before antagonist administration. This observation is consistent with data showing that ovulation occurs from the alternate ovary in subsequent cycles in greater than 85% of normal women (15, 16). The degree of prolongation of the follicular phase in the MFP studies is also consistent with new recruitment of a follicle, rather than resumption of growth of a follicle that was partially mature. Prior studies have shown that after surgical removal of a follicle or corpus luteum in women, ovulation occurs within 12–14 days (17). In previous studies in women in the MFP, 3 days of GnRH antagonist treatment resulted in gonadotropin suppression which was less complete than that achieved in the current studies and follicular phase prolongation of only 5–6 days (18), suggesting that less complete withdrawal of gonadotropin support than achieved in the current studies may halt follicular progress rather than result in follicular demise even in the MFP. The pronounced effect on follicular development in this stage of the cycle seen in both studies, despite the relatively minor changes in FSH induced by GnRH receptor blockade, is of particular interest. It is known that the follicle is exquisitely sensitive to FSH at this cycle stage; however, these results may suggest that LH is of greater importance at this time than previously believed.

Although subjects included in the LFP formed a more heterogeneous group, there was evidence that 72 h of gonadotropin withdrawal resulted in a temporary halt, but continued viability and regrowth of the dominant follicle when gonadotropins were again present. Previous studies in monkeys who received a GnRH antagonist on days 10–14 showed a delay in ovulation between 6–10 days (19), consistent with our findings. Taken together, our studies in the follicular phase suggest that the follicle is less tolerant to gonadotropin deprivation in the MFP than it is in the LFP, as indicated by the significantly greater prolongation of follicular phase length with GnRH antagonist administration in the MFP compared to the LFP; the increased time for estradiol to return to baseline in the MFP despite a similar time course for gonadotropins in the two groups, the inverse relationship found between the E_2 level on the first day of antagonist administration and the number of days to subsequent ovulation, and the ultrasound findings.

Seventy-two hours of gonadotropin deprivation in the MLP resulted in prompt luteolysis in all subjects, a precipitous decline in E_2 and P_4 levels, and the onset of vaginal bleeding. Previous studies of the dependence of the corpus luteum on gonadotropin support at various stages of its development have been somewhat contradictory. Early studies in monkeys indicated that GnRH antagonist administration in a dose that was sufficient to interrupt follicular dynamics had no effect on subsequent corpus luteum function when administered daily from day 1 after ovulation (20). However, subsequent studies with a more potent GnRH antagonist have indicated that a single day of GnRH antagonism in both the early and midluteal phases results in significant decreases in P_4, which are rapidly reversible in the macaque (7). In addition, withdrawal of pulsatile GnRH in hypothalamus-lesioned monkeys for 3 days resulted in decreased P_4 secretion and continued viability of the corpus luteum in studies conducted in the early and midluteal phases, but demise of the corpus luteum in studies in which GnRH was withdrawn in the late luteal phase (8).

In the human, studies have demonstrated both complete and incomplete luteolysis when GnRH antagonists were administered in the MLP in a single injection (9), but complete luteolysis with a longer duration or more complete suppression of gonadotropins (10, 21), as also seen in the current studies. Studies in the monkey (22) have indicated that luteolysis in response to GnRH antagonism was prevented by human menopausal gonadotropin (hMG), but not pure FSH. In humans, GnRH antagonist-induced luteolysis was overcome by hCG, but not hMG (10). As hMG contains predominantly FSH with a small amount of LH, both studies suggest that luteal function is much more dependent on LH than on FSH. As we were not able to achieve complete suppression of FSH, but near-total suppression of LH, the current studies would support the importance of LH and the relative lack of importance of FSH in the control of corpus luteum function. The cycle following antagonist-induced luteolysis was longer than the vehicle control cycle. Further study will be required to determine whether this difference is the result of altered gonadotropin dynamics after administration of the antagonist and possible effects of these on the developing follicle.

We have previously demonstrated a differential suppression of LH and FSH in the early follicular phase in response to GnRH antagonism over a range of single doses with two different GnRH antagonists, including the Nal-Glu GnRH antagonist (11, 23). The current studies indicate that this differential suppression of LH over FSH is maintained at all stages of the cycle with repeated administration of the Nal-Glu antagonist, that is with 72 h of GnRH receptor blockade. In general, the maximum percent suppression of LH with the Nal-Glu antagonist is greater in our studies than in other studies using similar doses of this same antagonist in postmenopausal and normal women and in men (9, 21, 24). These differences may relate to differences in our formulation of the drug, the route of administration, the physiological state of the subjects, or the characteristics of the LH assay used. For example, since α-subunit is suppressed to a lesser degree than LH after GnRH receptor blockade (11), the use of a LH assay with significant cross-reactivity to the free α-subunit (unlike our β-directed assay) would tend to underestimate the effect of GnRH receptor blockade on LH.

The decrease in FSH seen in the current studies is similar to that seen in a number of other human and animal studies (7, 9, 10, 21, 24, 25). FSH bioactivity, assessed by one of the two currently available FSH bioassays, is decreased to a slightly greater extent than immuno-FSH in women and men (26, 27) in response to GnRH antagonism, but the significance of this finding is unclear. The failure of a more profound suppression of either bio- or immunoactive FSH levels with up to 3 days of GnRH receptor blockade is continued evidence of some degree of GnRH-independent FSH secretion, at least over this limited period of time. Marked decreases in E_2 were present in all studies in response to GnRH antagonist administration, and the reduction in negative feedback of E_2 either at the pituitary or the hypothalamus may have played a part in the relative lack of sustained FSH suppression over the time period of this study. In addition, it is possible that inhibin levels were suppressed in response to the antagonist-induced decrease in LH, as seen in the luteal phase in previous studies (10). Loss of this restraining factor may also have contributed to a net decrease in FSH negative feedback after treatment with the antagonist. It is possible that a longer duration of antagonist administration would be associated with more marked decreases in plasma FSH levels through control of synthesis rather than secretion. Alternatively, other factors that stimulate FSH release, such as activin (28) or the putative FSH-releasing factor (29), may be entirely responsible for the FSH that is not suppressed by the GnRH antagonist in these studies.

We have assumed that any changes observed in response to administration of the Nal-Glu GnRH antagonist are the result of the changes in pituitary gonadotropin secretion produced. However, a direct effect on the ovary cannot be excluded, as human luteal homogenates (30) and granulosa cells (31) have been shown to possess specific binding sites for GnRH and its agonist analogs. In the rat, antagonist analogs of GnRH have been shown to inhibit luteal steroid production (32), but comparable findings have not yet been reported in the human.

In conclusion, these studies have shown that 72-h GnRH receptor blockade using a maximally suppressive dose of the Nal-Glu GnRH antagonist results in a greater suppression of LH than of FSH which is sustained over this time period. The follicle is less sensitive to this degree of gonadotropin withdrawal as it becomes progressively more mature from the MFP to the LFP. In the MLP, this degree of gonadotropin deprivation is not tolerated by the corpus luteum, and luteolysis occurs consistently. These findings add to our understanding of the complex interplay of hormonal and paracrine factors in the control of ovarian events. They also address dose and duration issues for subsequent studies of the use of GnRH antagonists to prevent premature LH surges in ovulation induction and in the design of novel contraceptive or contragestational agents.

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